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ORIGINAL ARTICLE

Chemical constituents from *Gazania linearis* cultivated in Egypt

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Abstract Chemical investigation of the cytotoxic *n*-hexane fraction of the rhizomes of *Gazania linearis* (Thunb.) Druce (Asteraceae), led to isolation of a steroid-glucoside and three triterpenes, together with three sesquiterpene lactones. The chemical structures of the isolated compounds were established based on their spectroscopic analyses (IR, 1D and 2D NMR, and mass spectroscopy) to be: lupeol-3-*O*-stearate (**1**), costunolide (**2**), 11,13-dihydro-costunolide (**3**), santamarine (**4**), in addition to lupeol (**5**), *β*-amyrin (**6**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**7**). The *n*-hexane fraction and compounds **4** and **6** displayed moderate cytotoxic activity toward MCF-7 and COLO-205 with IC_{50} values of 24.3, 34.0 μg/mL and 53.25 μM and 61.27, 21.10, and 25.70 μM, respectively compared to doxorubicin (IC_{50} 0.11 and 0.44 μM, respectively).

1. Introduction

Genus *Gazania* (family Asteraceae, tribe Arctotideae Cass. 1819) includes 25 herbaceous species native to South Africa.[1] They produce large, daisy-like composite flowers in brilliant shades of yellow and orange. They are often planted as a drought-tolerant groundcover.[2] Some species were cultivated in Egypt as ornamental plants among which *G. linearis*, *G. rigens*, and *G. nivea*.[3–5] Other species existing in Egypt, includes *G. hypridus*, *G. krebsiana*, *G. lichenstenii*, and *G. splendens*.[6] *Gazania* has been reported in folk medicine to prevent miscarriage and tooth ache, also it was incorporated in purgative preparations especially with aloes. Few studies have been reported to evaluate the biological effects of *Gazania*; which attributed antioxidant and hepatoprotective activities for *G. nivea* and antimicrobial activity for *G. rigens*.[7] *G. linearis* also called *G. longiscapa* DC, commonly known as treasure flower, is a mat-forming or clumping perennial herb growing from rhizomes.[2,8] Its leaves have long, winged petioles and form basal rosettes at the ground level around a branched stem. Leaves are linear to lanceolate, dull green with woolly undersides. The plant produces large, solitary daisy-like flower-heads in shades of bright yellow and orange colors. There is no report regarding the chemical constituents and biological activities of *G. linearis*. The present work reports the isolation and identification of lupeol-3-*O*-stearate (**1**), costunolide (**2**), 11,13-dihydro-costunolide (**3**), santamarine (**4**), lupeol (**5**), *β*-amyrin (**6**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**7**) (Fig. 1). In this study, compounds **1**–**4** were isolated here for the first time from the genus *Gazania*, while compounds **5**–**7** were isolated for the first time from the title plant. The *n*-hexane fraction and the isolated compounds **1**–**7** were evaluated for their cytotoxic activity.

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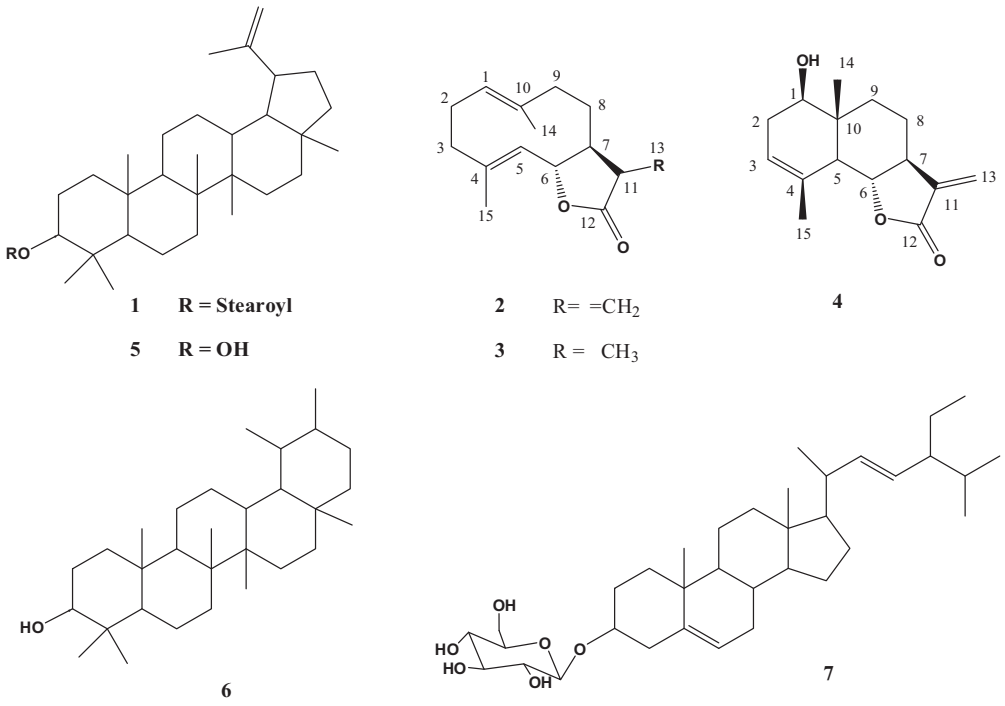

Figure 1 Compounds isolated form *G. linearis* **1**–**7**.

2. Experimental

2.1. General procedures

IR spectra were measured on Schimadzu Infrared-400 spectrophotometer (Japan). Electron impact mass spectrometry (EIMS) data were obtained with a JEOL JMS-700T mass spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Bruker DRX 400 spectrometer (Bruker, Rheinstetten, Germany). The GCMS was performed on Clarus 500 gas chromatography mass spetrometry (GCMS) (Perkin-Elmer, USA). The software controller/integrator was Turbo Mass, version 4.5.0.007 (Perkin-Elmer, USA). An Elite 5MS GC capillary column (30 mm × 0.25 mm × 0.5 μm, Perkin-Elmer, USA) was used. Helium, as a carrier gas (purity 99.9999%) was used at a flow rate of 2 mL/min (32 psi, flow initial 55.8 cm/s, split; 1:40). Temperature conditions were adjusted as follows: inlet line temperature, 200 °C; source temperature, 150 °C; trap emission, 100 °C and 70 eV electron energy. The column temperature program was: 50 °C for 5 min, increased to 220 °C (rate, 20 °C/min) and held for 5 min. The injector temperature was 220 °C. MS scan was from 50 to 700 *m*/*z*. Vacuum liquid chromatography (VLC) was carried out on silica gel 60 (0.04–0.063 mm, Merck, Darmstadt, Germany). Column chromatographic separations were performed over silica gel 60 (0.04–0.063 mm, Merck, Darmstadt, Germany) and TLC analyses were carried out on pre-coated silica gel F_{254} aluminum sheets plates (Merck, Darmstadt, Germany). Compounds were detected by spraying with *p*-anisaldehyde/H_2SO_4 reagent and heating at 110 °C for 1–2 min.

2.2. Plant material

Fresh roots of *G. linearis* were collected in March 2014 from cultivated plants at the medicinal plants farm of Faculty of Pharmacy, Al-Azhar University, Assiut branch. The plant was identified and authenticated by Prof. Dr. Salah M. El-Nagar, Professor of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen (G-3\2014) was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut branch.

2.3. Extraction and isolation

The shade-dried roots (1 kg) were crushed and macerated with MeOH (3 × 2.5 L, 72 h, each) at room temperature. The combined extract was concentrated under reduced pressure to afford a dark brownish residue (17.2 g). The latter was suspended in distilled water (100 mL) then partitioned exhaustively and subsequently with *n*-hexane, $CHCl_3$ and EtOAc (4 × 300 mL, each). Each fraction was concentrated under reduced pressure to give *n*-hexane (5.4 g), $CHCl_3$ (2.7 g), EtOAc (1.9 g), and aqueous (6.1 g), fractions. The *n*-hexane fraction (5.4 g) was subjected to vacuum liquid chromatography (VLC), over silica eluted with *n*-hexane:EtOAc gradient to yield 4 fractions. Fraction 1 (2.1 g) was subjected to silica gel column chromatography eluted with *n*-hexane:EtOAc (98:2 to 85:15) to afford five fractions:HGL-1 to HGL-5. Silica gel column chromatography (40 g, 50 × 2 cm) of fraction HGL-1 (195 mg) using *n*-hexane/EtOAc gradient gave

Table 1 ^{1}H NMR data of compounds **2**–**4** (400 MHz, $CDCl_3$).

No.	**2** δ_H [mult., J (Hz)]	**3** δ_H [mult., J (Hz)]	**4** δ_H [mult., J (Hz)]
1	4.83 (1H, m)	4.84 (1H, m)	3.66 (1H, dd, J = 10.2, 6.6 Hz)
2	2.0–2.35 (1H, m)	1.98–2.34 (1H, m)	2.38 (1H, m)
	1.68 (1H, m)	1.65 (1H, m)	2.01 (1H, m)
3	2.0–2.35 (1H, m)	1.98–2.34 (1H, m)	5.35 (1H, m)
	2.43 (1H, m)	2.45 (1H, m)	
5	4.72 (1H, d, J = 9.6 Hz)	4.71 (1H, m)	2.33 (1H, br.d. J = 11.2 Hz)
6	4.57 (1H, t, J = 8.4 Hz)	4.53 (1H, m)	3.95 (1H, t, J = 11.4 Hz)
7	2.56 (1H, t, J = 7.8 Hz)	2.51 (1H, m)	2.50 (1H,m)
8	2.0–2.35 (2H, m)	1.98–2.34 (2H, m)	2.11 (1H,m,)
			1.65 (1H, m)
9	2.0–2.35 (2H, m)	1.98–2.34 (2H, m)	2.06 (1H, m)
			1.32 (1H, m)
11	–	2.51 (1H, m)	–
13	6.25 (1H, d, J = 2.9 Hz)	1.24 (3H, d, J = 7.1 Hz)	6.06 (1H, d, J = 3.5 Hz)
	5.51 (1H, d, J = 2.9 Hz)		5.41 (1H, d, J = 3.5 Hz)
14	1.41 (3H, s)	1.39 (3H, s)	0.87 (3H, s)
15	1.69 (3H, s)	1.68 (3H, s)	1.83 (3H, s)

compound **1** (17 mg). Fraction HGL-2 (327 mg) was chromatographed over silica gel column (50 g, 50 × 2 cm) using *n*-hexane/EtOAc gradients to obtain compounds **2** (5.3 mg), **3** (7.3 mg), and **4** (4.8), in addition to a mixture of compounds still under further investigation. Silica gel column chromatography (80 g, 50 × 2 cm) of fraction HGL-3 (112 mg) using *n*-hexane/EtOAc gradient afforded compounds **5** (22.5 mg) and **6** (18.9 mg). Similarly, fraction HGL-5 (205) was treated as fraction HGL-3 to obtain compound **7** (78 mg).

2.4. Alkaline hydrolysis

A solution of **1** (8 mg) in 3% KOH/MeOH (4 mL) was left to stand for 15 min at room temperature then neutralized with 1 N HCl/MeOH. The solution was extracted with $CHCl_3$. The solvent was evaporated and the residue obtained was chromatographed on a silica gel column using *n*-hexane: EtOAc gradient to furnish lupeol and methyl ester of stearic acid. Lupeol was identified by EIMS analysis and co-TLC with authentic sample. The methyl ester of stearic acid was identified by GCMS.[9]

2.5. Cytotoxic activity

The cytotoxicity was evaluated by the [3H] thymidine assay using breast cancer (MCF-7) and colon cancer (COLO-205) cell lines.[10] Doxorubicin was used as a positive control. The cell lines were obtained from Al-Azhar University Centre for Viral Research, Cairo, Egypt.

3. Results and discussion

Compound **1** was isolated as white amorphous powder. The analysis of its spectral data assigned a lupine skeleton as identified by the exomethylene signals at δ_H 4.56 (H-29A) and 4.68 (H-29B)/δ_C 109.3 (C-29) and 151.0 (C-20), in addition to, seven tertiary methyl signals at δ_H 0.80, 0.83, 0.84, 0.93, 0.97, 1.07, and 1.69, respectively, together with the absorption bands at 1657, 884 cm^{-1} (exocyclic *di*-substituted double bond), 1370 and 1380 cm^{-1} (geminal dimethyl) in IR spectrum.[11–13] In the ^{1}H and ^{13}C NMR spectra, the terminal methyl signal at δ_H 14.1\δ_C 0.88 (t, J = 6.7 Hz, H-18), multiplet methylene at δ_H 2.38 (H-2\), and methylene signals at δ_H 1.26–1.29/δ_C 29.4–29.7, together with the ester carbonyl at δ_C 170.2 indication on the presence of a fatty acid residue (Tables 1 and 2). The molecular ion peak at m/z 692 $[M]^+$ in the EIMS spectrum, with significant ion peaks at m/z 677 $[M-CH_3]^+$ and 410 $[M-C_{18}H_{36}O_2+H]^+$, accounted for stearyl ester of lupeol, which was confirmed by alkaline hydrolysis followed by GCMS analysis of the fatty acid methyl ester. According to these data and by comparison with literature,[11,14,15] compound **1** was identified as lupeol-3-*O*-stearate. This is the first isolation of this compound from the genus *Gazania*.

Table 2 ^{13}C NMR data of compounds **2**–**4** (100 MHz, $CDCl_3$).

No.	**2**	**3**	**4**
1	126.8	127.4	75.2
2	28.2	28.4	32.8
3	41.1	41.1	121.5
4	141.1	137.1	133.5
5	127.6	127.1	51.1
6	81.9	81.6	81.6
7	50.8	54.5	51.0
8	26.3	26.1	21.2
9	39.6	39.5	34.4
10	137.2	140.0	40.9
11	141.5	42.4	139.1
12	170.7	178.6	170.8
13	120.2	13.4	116.9
14	16.2	16.3	11.2
15	17.5	17.4	23.5

Compound **2** was isolated as white crystals. The EIMS analysis showed a molecular ion peak at m/z 232 $[M]^+$. The IR spectrum showed absorption bands at 1761 (γ-lactone) and 860 cm^{-1} (tri-substituted double bond). The two doublet

signals at δ_H 5.51 and 6.25 (J = 2.9 Hz, each) in the ^{1}H NMR spectrum (Table 1), revealed the presence of exocyclic methylene. Moreover, two singlet methyls at δ_H 1.41 (H-14) and 1.69 (H-15), two olefinic protons at δ_H 4.83 (1H, m) and 4.72 (d, J = 9.6 Hz), in addition to two triplet signals at δ_H 4.57 (J = 8.4 Hz) and 2.56 (t, J = 7.8 Hz) were observed. The ^{13}C NMR (Table 2) showed the presence of 15 carbons; two methyls, five methylenes, four methines, and four quaternary carbons. The γ-lactone moiety as suggested by the IR spectrum was confirmed by the four olefinic carbons associated with two double bonds at δ_C 141.1/127.6 and 126.8/137.2 and the carbon resonances at δ_C 170.7, 141.5, 81.9, and 50.8. The sequence of the aliphatic and olefenic protons was assigned using the homonuclear correlation spectroscopy (COSY) experiment (Fig. 2), which afforded the series from H-1 to H-3 and from H-5 to H-9. The heteronuclear multiple bond correlation (HMBC) cross peaks of H-1 with C-10, H-3 with C-4, H-9 with C-10, H-5 with C-6 and C-4, and H-15 with C-4 indicated a costunolide nucleus. From the above mentioned data, **2** was identified as costunolide which was first isolated from *Saussurea costus* roots.[16] Also, it was reported from several plants as bay leaf,[17,18] *Rudbeckia* species,[19] and *Magnolia grandiflora*.[20] It was isolated here for the first time from the genus *Gazania*.

Compound **3** was isolated as yellowish resinous residue. The EIMS spectrum showed a molecular ion peak at m/z 234 $[M]^+$. The NMR data of **3** were similar to those of **2**, except the appearance of doublet signal for a secondary methyl group at δ_H 1.24 (3H, J = 7.1 Hz) and a multiplet methine proton at δ_H 2.51 (1H, m) and disappearance of the exomethylene signals. Together with increment of 2 mass units, compound **3** was assigned to be a dihydro-derivative of **2**. The 2D correlations of the secondary methyl with C-7 and C-12 allowed its attachment at C-11. Accordingly, compound **3** was confirmed to be 11,13-dihydrocostunolide.[21] It was isolated here for the first time from the genus *Gazania*.

Compound **4** was isolated as white crystals. The EIMS showed molecular ion peak at m/z: 248 $[M]^+$, in addition to fragment ion peak at m/z 230 $[M-H_2O]^+$. The IR spectrum showed absorption bands ascribable to hydroxyl, γ-lactone, and *tri*-substituted double bond (3480, 1751, and 855 cm^{-1}, respectively). The ^{1}H and ^{13}C NMR spectra showed signals assignable to two tertiary methyls at δ_H 0.87 (3H, s, Me-14) and 1.83 (3H, s, Me-15), two oxymethines at δ_H 3.66 (1H, dd, J = 10.2, 6.6 Hz) and 3.95 (1H, t, J = 11.4 Hz), an olefinic proton signal at δ_H 5.35 (1H, m, H-3), and an exomethylene group at δ_H 6.06 and 5.41 (1H, d, J = 3.5 Hz, each) (Table 1). The ^{13}C NMR showed the presence of 15 carbons: two methyls, four methylenes, five methines, and four quaternary carbons. In addition, the two olefinic carbons of a double bond at δ_C 121.5/133.5 and carbon resonances at δ_C 170.8, 139.1, 81.6, and 51.1 confirmed the γ-lactone moiety as suggested by the IR spectrum (Table 2). The COSY experiment afforded two spin systems from H-1 to H-3 and from H-5 to H_2-9 (Fig. 2). The HMBC spectrum showed cross peaks between the hydroxymethine (H-1) and C-10, H-3 and Me-15 with C-4, H-5 with C-4 and C-7, H-9 with C-10, and Me-14 with C-10, C-1, and C-9. In addition, HMBC cross peaks of H-7 (δ_H 2.50) to the lactone carbonyl carbon (C-12) and oxygenated methine at δ_H 3.95 (H-6) to the quaternary carbon at δ_C 139.1 (C-11) were observed. From the above mentioned data, compound **4** was identified as 1-hydroxy-eudesmanolide. The NMR data are in good agreement with those reported for santamarine,[20,22] which was previously isolated from *Laurus nobilis* L.,[23] roots of *Costus speciosus*,[22] and flowers of *Tanacetum vulgare*.[24] This is the first isolation of this compound from the genus *Gazania*.

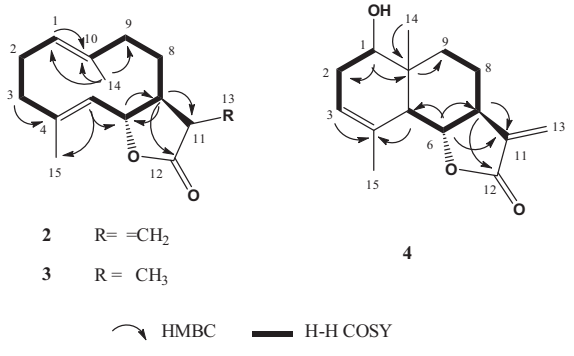

Figure 2 Key 2D correlations of compounds **2**–**4**.

Table 3 Cytotoxic activity results.

Sample	(IC_{50} μM)	
	MCF-7	COLO-205
n-Hexane fraction[a]	24.3 ± 1.30	34.0 ± 2.40
1	>144.50	>144.50
2	>431	>431
3	>427.40	>427.40
4	53.25	61.27
5	>235	>235
6	21.10	25.70
7	>174	>174
Doxorubicin	0.11	0.44

[a] IC_{50} μg/mL.

The cytotoxic effect of obtained fractions and the isolated compounds were tested against MCF-7 and COLO-205 cancer cell lines. It was found that *n*-hexane fraction and compounds **4** and **6** showed moderate cytotoxic activity toward MCF-7 and COLO-205 with IC_{50} values of 24.3, 34.0 μg/mL and 53.25 μM and 61.27, 21.10, and 25.70 μM, respectively compared to doxorubicin (IC_{50} 0.11 and 0.44 μM, respectively). Other fractions and compounds had no activity (Table 3).

Compounds **5**–**7** were identified as lupeol,[25] β-amyrin,[26] and β-sitosterol-3-*O*-β-D-glucopyranoside,[27] respectively by comparison of their physical and spectral data with literature. These compounds are isolated here for the first time from the title plant.

Conflict of interest

None declared.

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